

TNF/TNFR Working Party Distribution List for October 18, 1995 Team Meeting

SB

Edward R Appelbaum
M. Burke
E. Dul
Edward R Gimmi
Don E Griswold
Jeremy A Harrop
Yen Sen Ho-1
Mark R Hurle
John C Lee
Sally D Lyn
Gordon P Moore
Terence Porter
Manjula P Reddy
Carol Silverman
Kong B Tan
Ping Tsui-1
X. Wang
Peter R Young
T. L. Yue

HGS

Reiner Gentz
Jian Ni
Guo-Liang Yu
Susan Lackey

SB cc: List

Sherin S Abdel-Meguid
Christine M Debouck
Giora Z Feuerstein
George I Glover
Maxine Gowen
C Preston Hensley
Zdenka L Jonak
Kristy Kikly-1
Loretta C McCauley
Jo Anne Mackey
Andrew J Nichols
Martin Rosenberg
Allan R Shatzman
Theodore J Torphy

Document Management UW 2309

BEST AVAILABLE COPY

TNF/TNFR Working Party Distribution List for October 18, 1995 Team Meeting

SB

Edward R Appelbaum
M. Burke
E. Dul
Edward R Gimmi
Don E Griswold
Jeremy A Harrop
Yen Sen Ho-I
Mark R Hurdle
John C Lee
Sally D Lyn
Gordon P Moore
Terence Porter
Manjula P Reddy
Carol Silverman
Kong B Tan
Ping Tsui-I
X. Wang
Peter R Young
T. L. Yue

HGS

Reiner Gentz
Jian Ni
Guo-Liang Yu
Susan Lackey

SB cc: List

Sherin S Abdel-Meguid
Christine M Debouck
Giora Z Feuerstein
George I Glover
Maxine Gowen
C Preston Hensley
Zdenka L Jonak
Kristy Kikly-I
Loretta C McCauley
Jo Anne Mackey
Andrew J Nichols
Martin Rosenberg
Allan R Shatzman
Theodore J Torphy

Document Management UW 2309

Ruben EXHIBIT 2071
Ruben v. Wiley et al.
Interference No. 105,077
RX 2071

TNF/TNFR Working Party: Minutes of October 18, 1995 SB/HGS Joint Meeting
Meeting held at Upper Merion on October 18, 1995.

Attendee:

SB: E. Appelbaum, M. Burke, E. Dul, J. Harrop, M. Hurle, Z. Jonak, K. Krikly, J. Lee, S. Lyn, G. Moore, M. Reddy, K.B. Tan, P. Tsui, A. Trunch, P. Young, X. Wang, T.L. Yue

HGS: R. Gentz, J. Ni, G-L. Yu,

P. Young presented a critical path depicting key components a strategy for identification of gene function for TNF/TNFR related genes (overhead PY1).

M. Hurle reviewed the sequences of 8 novel TNFR like and 3 TNF like genes (overheads MH1-6).

J. Ni reviewed for the group the current members of known TNF and TNFR superfamily, as well as the recently described associated proteins. Many of the known proteins are present in the HGS database as full length genes. The rat Fas ligand like molecule has been expressed in E. coli and polyclonal antibody is generated. The second TNF like ligand, termed TNF γ or EDAP, is being pursued by HGS as a novel antiviral agent. This molecule has been expressed and purified at HGS. The third TNF like ligand has been shown to be expressed in most tissues with the highest expression in the heart. Of the eight novel TNFR like proteins, one appears to be a soluble receptor to which antibody has been made. HGS is more interested in working on the ligands than the receptors.

K.B. Tan reviewed the tissue/cell distribution of the 5 most interesting TNF/TNFR genes. The results are summarized in overheads KB1-8. Most of these genes (TR2, TR3 and TL1) appear to be primarily expressed in hematopoietic cells while TR1 message was found in an osteoclast cell line. A larger size message for TR1 was also found in a hematopoietic precursor and a T cell line.

P. Young/S. Lynn provided an update on the efforts of cloning and expressing what is now known as TR2. Expression of the human IgG1 Fc-fusion protein in mammalian cells is successful. Monoclonal antibody production has been initiated.

E. Appelbaum reported the expression of the rat Fas ligand homolog in two fusion constructs as epitope tagged proteins.

A number of issues surfaced during the ensuing discussion period and they are listed below with solutions and/or action plans:

1. *Nomenclature*

Given that there are multiple designations of the original ESTs by HGS and subsequent names/numbers given by SB/ATG and the increasing number of interesting molecules, it has become apparent that a unified nomenclature should be established. It was agreed all the novel TNF/TNFR related full length genes will have T as the first prefix to denote their homology to these superfamilies, followed by L or R to denote membership to the ligand or receptor superfamily, followed by a unique number for each unique full length novel sequence. Splice variants will be sub-classified with an alphabet; e.g. TR2a for HLHAB49 (see also post meeting Tables 1 and 2 in AT1-2 for details).

2. *Focus*

HGS will focus on putative ligands and SB on the receptors

3. *Sequence errors/differences between HGS and SB*

Consultation among the two parties is recommended before any work is to be done. Any discrepancies in sequence that may have impact on patents must seek legal input.

4. *Spliced variants*

Whether spliced variants are meaningful targets would require further confirmation by PCR of the inserts and TNT tests should be conducted.

5. *Reagent for exchange*

Currently, the following tool reagents are available:

SB: TR2-Ig

HGS: TL1 and TR1 proteins and antisera to TL2 and TR1

6. *Coordination of expression efforts*

SB will concentrate on expressing all TR molecules and some TL proteins

HGS will express all TL proteins

7. *Third party MTA*

Established through HGS:

a. Alan Porter (U. Singapore) on TR2 (signal transduction) and TRAP clones (2 hybrid screen)

b. Kwon (Indiana U.) on TR2 (genomic cloning and spliced variant)

Pending at HGS:

a. Aggarwal (MD Anderson) on TR1, TL1 etc (biological effects, functions and effect on tumor cells)

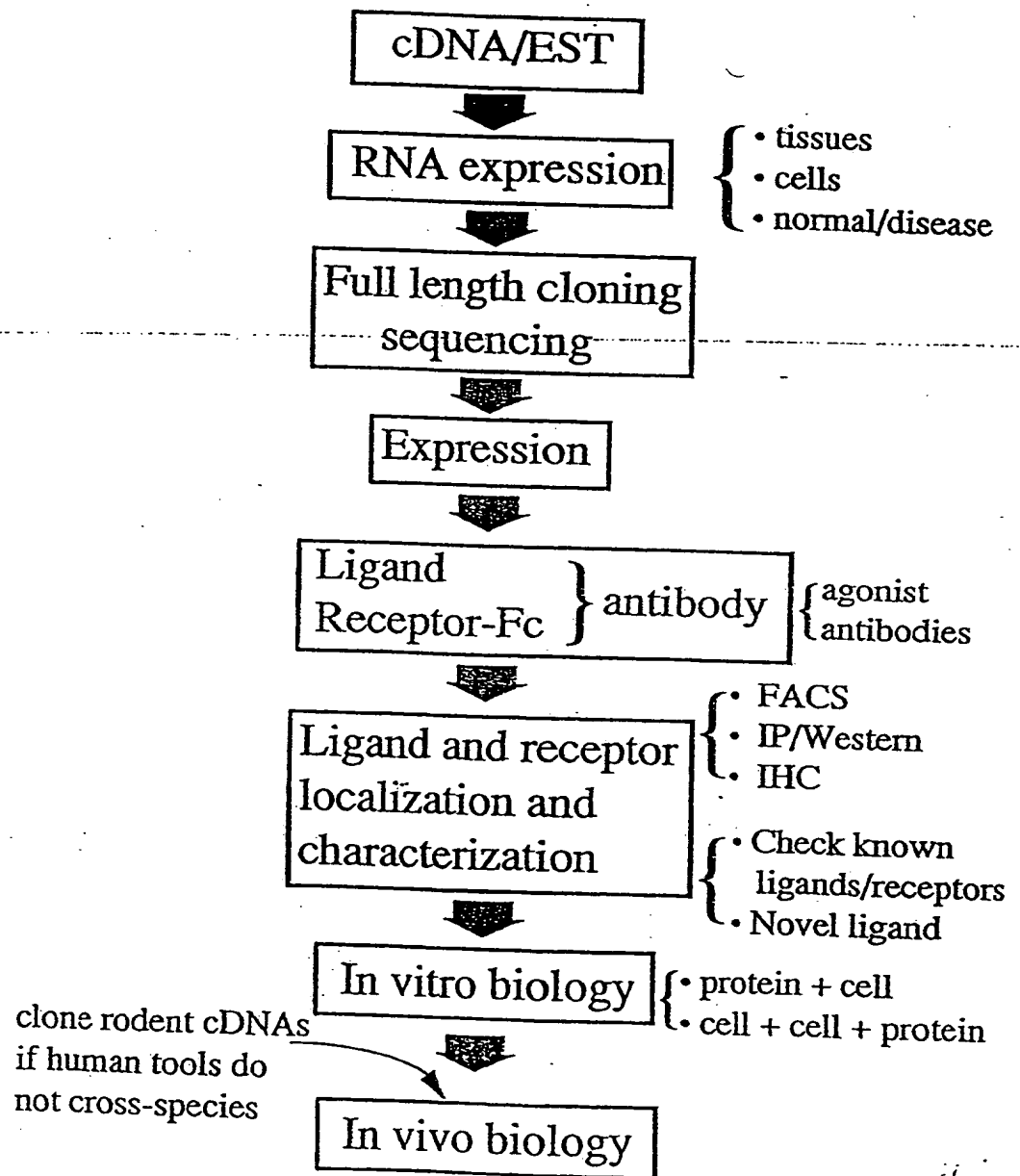
b. Dormer (Indiana U.) on novel TRAP molecules

Underdiscussion at SB:

a. Beutler (UTSW) on novel TRs (signaling mechanisms)

b. Kollias (Hellenic Pasteur institute) on novel TIs (transgenic/knockout)

Critical path



Novel TNF Receptor Homologs

Molecule	Project ID	#Clones	#ESTs	Homolog	% Identity	Length
TR1	HSABH13	5	11	human TNFR-II	28%	402aa
TR2	HLHAB49	15	58	mouse CD40	29%	284aa
TR2a	HHFF179	3	18	(splice of TR2)		
TR2b	HMSBZ59	2	17	(splice of TR2)		
TR2c	HROAG75	1	2	(splice of TR2)		
TR3	HTTBN61	3	5	human TNFR-I	37%	71aa (extracellular)
TR4	HSAYO45	1	3	mouse TNFR-I	48%	79aa (death domain)
TR5	HTXEI33	1	4	human 4-1BB	100%	255aa (splice)

Other non-full length clones (class 2) in ATG/HGS database not listed above*

TNFR I 2
 TNFR II 1
 OX40 2
 Fas 2
 CD40 4
 CD27 1
 CrmB 1
 4-1BB 1

* sequence analysis in progress

Novel TNF (Ligand) Homologs

<u>Molecule</u>	<u>Project ID</u>	<u>#Clones</u>	<u>#ESTs</u>	<u>Homolog</u>	<u>% Identity</u>	<u>Length</u>
TL1	HUVEO91	4	29	human TNF α	30%	216aa
TL2	HTPAN08	17	36	rat Fas ligand	27%	125aa
TL3	HLTBT71	12	28	rat TNF α	27%	120aa
TL4	HPDD012					

Other non-full length clones (class 2) in ATG/HGS database not listed above*

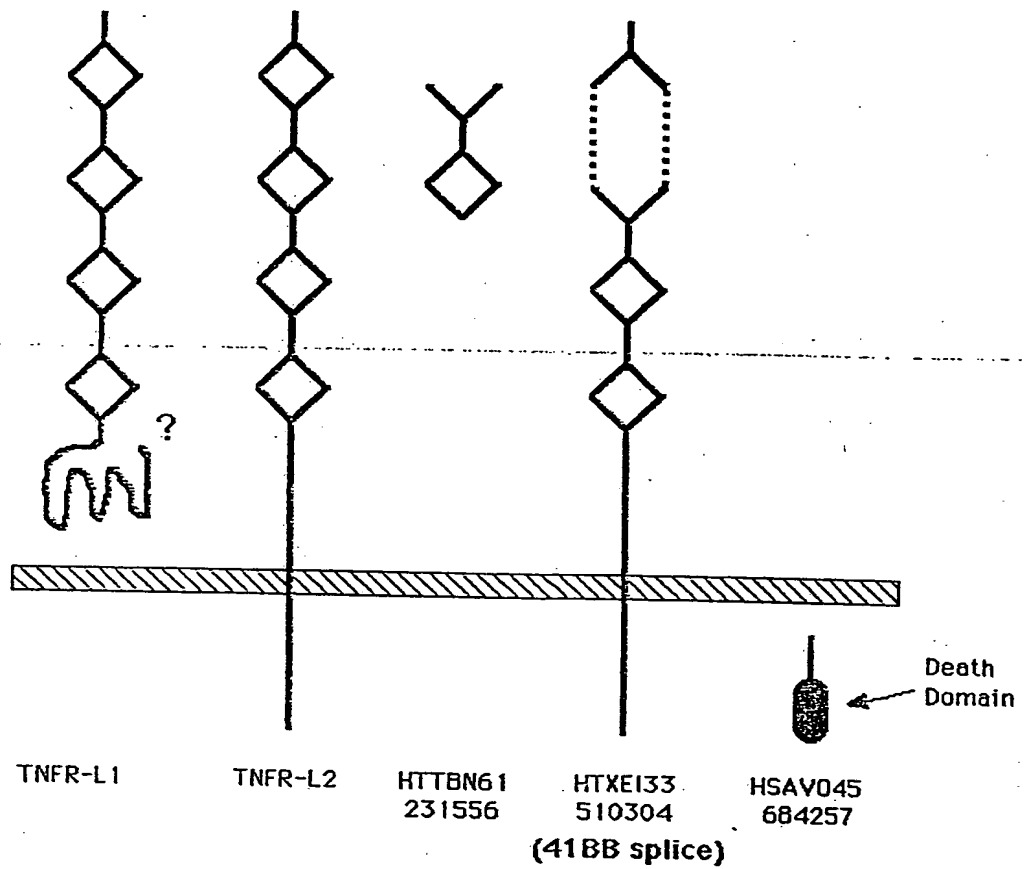
LT- α 2
 LT- β 1
 Fas Ligand 3
 CD30 Ligand 2

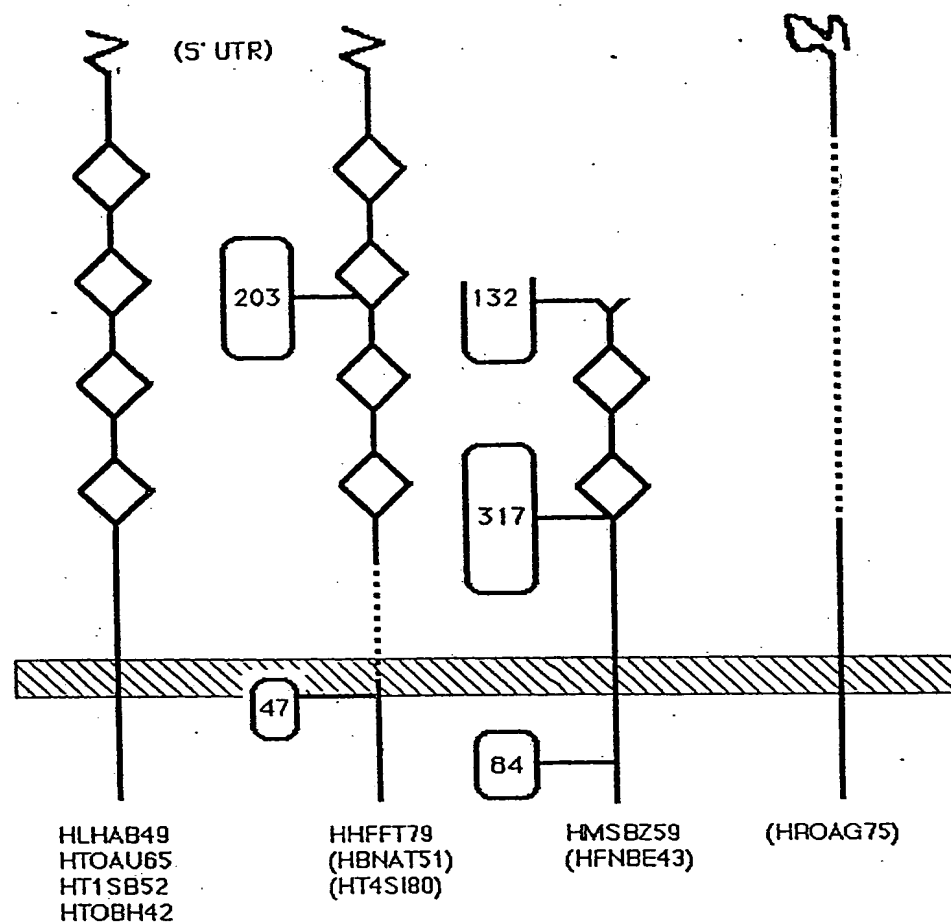
* sequence analysis in progress

Novel TNF Receptors and Ligands

Molecule	Project ID	#Clones	#ESTs	Homolog	% Identity	Length
TNFR-L1	HSABH13	5	11	human TNFR-2	28%	402aa
TNFR-L2	HLHAB49	15	58	mouse CD40	29%	284aa
TNFR-L2a	HHFT79	3	18	(splice of TNFR-L2)		
TNFR-L2b	HMSBZ59	2	17	(splice of TNFR-L2)		
TNFR-L2c	HROAG75	1	2	(splice of TNFR-L2)		
TNFR-L3	HTTBN61	3	5	human TNFR-1	37%	71aa (extracellular)
TNFR-L4	HSAVO45	1	3	mouse TNFR-1	48%	79aa (death domain)
41BB-L1	HTXEI33	1	4	human 4-1BB	100%	255aa (splice)
<hr/>						
TNF	HUVEO91	4	29	human TNF α	30%	216aa
TNF	HTPAN08	17	36	rat Fas ligand	27%	125aa
TNF	HLTBT71	12	28	rat TNF α	27%	120aa

Novel TNF Receptor-Related Molecules



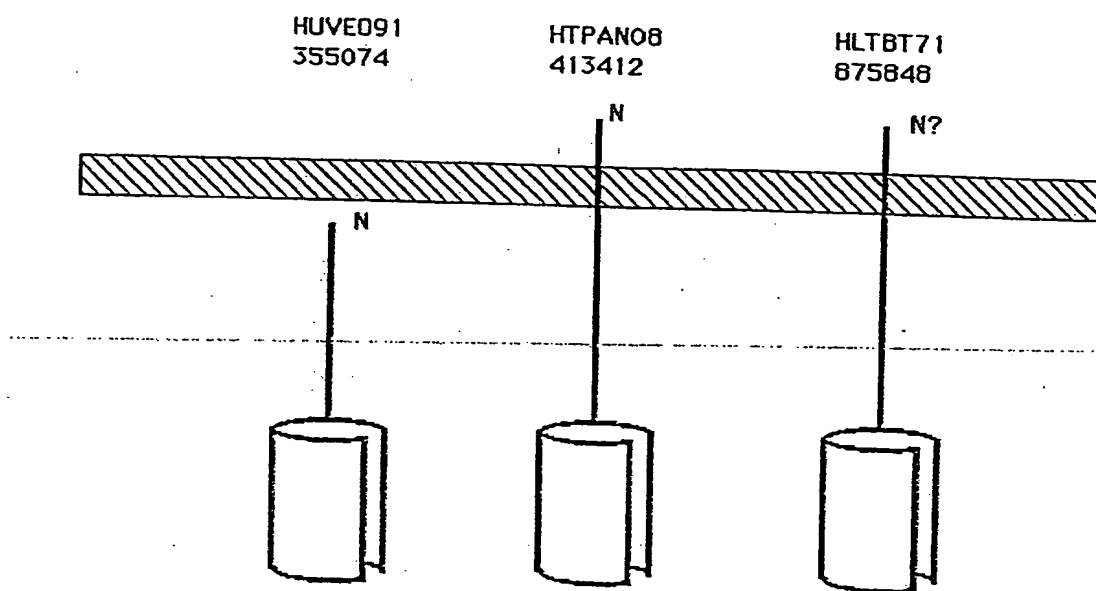


All solid lines have sequence in common

Sizes of splice inserts are shown inside oval-shaped symbol

Project IDs for full length sequences or (individual ESTs) are shown

Novel TNF-Related Molecules



Summary of Receptor Expression

- Sequenced HTOAU65 (103902)
 - Named this TNFRL2
 - TNFRL2 differs from patent
 - Position 41 (Ser to Phe)
 - Position 17 is Arg (Lys in some ESTs)
 - one silent change
- Cloned into mammalian expression vector COSFc
 - Re-engineered start codon
- Expressed Human IgG1 Fc-fusion TNFRL2
 - COS cells
 - CHO-E1a
- Monoclonal antibody production initiated
- Sequenced HSABH13(195197)
 - Named this TNFRL1
 - Sequence different from patent
 - C 1077 missing
 - Altered C-terminus and an 11 aa longer protein

325532 TGAAGTTCCTC TGCTGGAGTT CATCTCTGCTA GCTGGGTTCC CGAGCTGCCG
103902
Tnfr12fctextcontig_1 CTATATAAGC AGAGCTGGGT ACGTGAACCG TCAGATCGCC TGGAGAAGCC

251
338804 ATGGA GCCTCCTGGA GACTGGGGGC CTCTCCCTG 300
338805 GTCTGAGCCT GAGGCATGGA GCCTCCTGGA GACTGGGGGC CTCTCCCTG
Tnfr12 CCT GAGGCATGGA GCCTCCTGGA GACTGGGGGC CTCTCCCTG
355073 CCT GAGGCATGGA GCCTCCTGGA GACTGGGGGC CTCTCCCTG
325531 GTCTGAGCCT GAGGCATGGA GCCTCCTGGA GACTGGGGGC CTCTCCCTG
325532 GTCTGAGCCT GAGGCATGGA GCCTCCTGGA GACTGGGGGC CTCTCCCTG
103902
Tnfr12fctextcontig_1 ATCGAATTGG CAGCCATGGA GCCTCCTGGA GACTGGGGGC CTCTCCCTG

301 Lys 350
338804 GAGATCCACC CCCAAAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACT
338805 GAGATCCACC CCCAAAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACT
Tnfr12 GAGATCCACC CCCAAAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACT
355073 GAGATCCACC CCCAAAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACT
325531 GAGATCCACC CCCAAAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACT
325532 GAGATCCACC CCCAAAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACT
103902
Tnfr12fctextcontig_1 GAGATCCACC CCCAAAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACT

351 Ser 400
338804 TCCTGGGAGC CCCCTGCTAC GCCCCAGCTC TGCCGTCCTG CAAGGAGGAC
338805 TCCTGGGAGC CCCCTGCTAC GCCCCAGCTC TGCCGTCCTG CAAGGAGGAC
Tnfr12 TCCTGGGAGC CCCCTGCTAC GCCCCAGCTC TGCCGTCCTG CAAGGAGGAC
355073 TCCTGGGAGC CCCCTGCTAC GCCCCAGCTC TGCCGTCCTG CAAGGAGGAC
325531 TCCTGGGAGC CCCCTGCTAC GCCCCAGCTC TGCCGTCCTG CAAGGAGGAC
325532 TCCTGGGAGC CCCCTGCTAC GCCCCAGCTC TGCCGTCCTG CAAGGAGGAC
103902
Tnfr12fctextcontig_1 TCCTGGGAGC CCCCTGCTAC GCCCCAGCTC TGCCGTCCTG CAAGGAGGAC

401 Phe 450
338804 GAGTACCCAG TGGGCTCCGA GTGCTGCCCC AAGTGCAGTC CAGGTTATCG
338805 GAGTACCCAG TGGGCTCCGA GTGCTGCCCC AAGTGCAGTC CAGGTTATCG
Tnfr12 GAGTACCCAG TGGGCTCCGA GTGCTGCCCC AAGTGCAGTC CAGGTTATCG
355073 GAGTACCCAG TGGGCTCCGA GTGCTGCCCC AAGTGCAGTC CAGGTTATCG
325531 GAGTACCCAG TGGGCTCCGA GTGCTGCCCC AAGTGCAGTC CAGGTTATCG
325532 GAGTACCCAG TGGGCTCCGA GTGCTGCCCC AAGTGCAGTC CAGGTTATCG
103902 AATTCC CCCGGGNGGA GTGCTGCCCC AAGTGCAGTC CAGGTTATCG
Tnfr12fctextcontig_1 GAGTACCCAG TGGGCTCCGA GTGCTGCCCC AAGTGCAGTC CAGGTTATCG

451 500
338804 TGTGAAGGAG GCCTGCGGGG AGCTGACGGG CACAGTGTGT GAACCTTGCC
338805 TGTGAAGGAG GCCTGCGGGG AGCTGACGGG CACAGTGTGT GAACCTTGCC
Tnfr12 TGTGAAGGAG GCCTGCGGGG AGCTGACGGG CACAGTGTGT GAACCTTGCC
355073 TGTGAAGGAG GCCTGCGGGG AGCTGACGGG CACAGTGTGT GAACCTTGCC
325531 TGTGAAGGAG GCCTGCGGGG AGCTGACGGG CACAGTGTGT GAACCTTGCC
325532 TGTGAAGGAG GCCTGCGGGG AGCTGACGGG CACAGTGTGT GAACCTTGCC
103902
Tnfr12fctextcontig_1 TGTGAAGGAG GCCTGCGGGG AGCTGACGGG CACAGTGTGT GAACCTTGCC

501 550
338804 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGTCTGCAG
338805 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGTCTGCAG
Tnfr12 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGTCTGCAG
355073 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGTCTGCAG
325531 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGTCTGCAG
325532 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGTCTGCAG
103902
Tnfr12fctextcontig_1 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGTCTGCAG

551 500
338804 TGCCCAATGT GTGACCCAGC CATGGGCTTG CGCGCGAGCC GGAAGTCTC

Sally Lynn

GAP of: Tnfr12.Pep che 4189 from: 1 to: 283

TRANSLATE of: tnfr12.seq check: 9389 from: 9 to: 857
generated symbols 1 to: 283.

TNF receptor like 2 sequence was copied from HGS full length report
filename 103902.000, HGS code 103902, Project ID: HTLSB52

REFORMAT of: Tnfr12.Seq check: -1 from: 1 to: 881 May 16, 1995 13:54
(No documentation) . . .

to: Tnfr12fclink.Pep check: 9984 from: 1 to: 432

TRANSLATE of: tnfr12fclink.seq check: 768 from: 665 to: 1960
generated symbols 1 to: 432.

Tnfr12fclink is sequenced 103902fc (EcoRI Asp718 of
Tnfr12fcContig1.seq) in Cosfclink.

COSfclink is SFcr1cos4 with the hinge CH2CH3 region of human IgG
inserted in place of the FcR cDNA and a 27bp linker inserted upstream . . .

Symbol comparison table: Gencoredisk: [Gcgcore.Data.Rundata]Nwsgappp.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	315.8	Length:	439
Ratio:	1.116	Gaps:	6
Percent Similarity:	85.145	Percent Identity:	76.812

Tnfr12.Pep x Tnfr12fclink.Pep August 11, 1995 10:46 ..

```

      1 MEPPGDWGPFPWRSTPRTDVLRLVLYLTFLGAPCYAPALHS KEDEYYPVG 50
      |||
      1 MEPPGDWGPFPWRSTPRTDVLRLVLYLTFLGAPCYAPALHS KEDEYYPVG 50
      |||
      51 SECCPKCSPGYRVKEACGELTGTVCPCPPGTYIAHLNGLSKLQCCMC 100
      |||
      51 SECCPKCSPGYRVKEACGELTGTVCPCPPGTYIAHLNGLSKLQCCMC 100
      |||
      101 PAMGLRASRNCSTENAVCGCSPGHFCTVQGDHCAACRAYATSSPGQRV 150
      |||
      101 PAMGLRASRNCSTENAVCGCSPGHFCTVQGDHCAACRAYATSSPGQRV 150
      |||
      151 QKGGTESQDTLCQNCPPGTFFSPNGTLEECQHQTCSWLVTKA....GAGT 196
      |||
      151 QKGGTESQDTLCQNCPPGTFFSPNGTLEECQHQTCSWLVTKARSIEGRGT 200
      |||
      197 S.....SSHWVWFLSGSLVIVIVCSTVGLIICV.. 225
      |||
      201 EPKSADKTHTCPPCPAPELLGGPSVFLFPKPKDTLMSRTPEVTCVVVD 250
      |||
      225 .....KRRKPRGD.....VVKVIVSVQKQOE 247
      |||
      251 VSHEDPEVKPMWYVDGVEVNAKTKPREEQYNSTYRVVSVLTVLHQDMLN 300
      |||
      242 AEGETAVI..EALQAPPDVTVAVEETIPSFTGRSPNH..... 283
      |||
      301 GKEYKCKVSKALPAP.....TEKTISKAKGQPREPQVYTLPPSRDEL 343
      |||
```

2 extra Cys

Sally Lyn

PY4

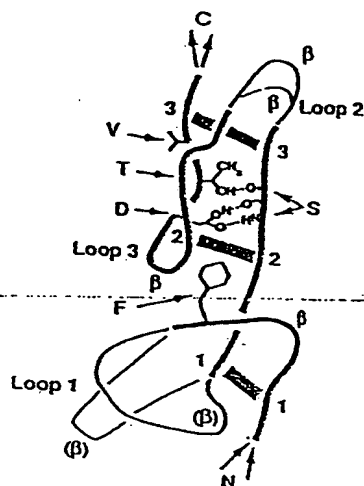
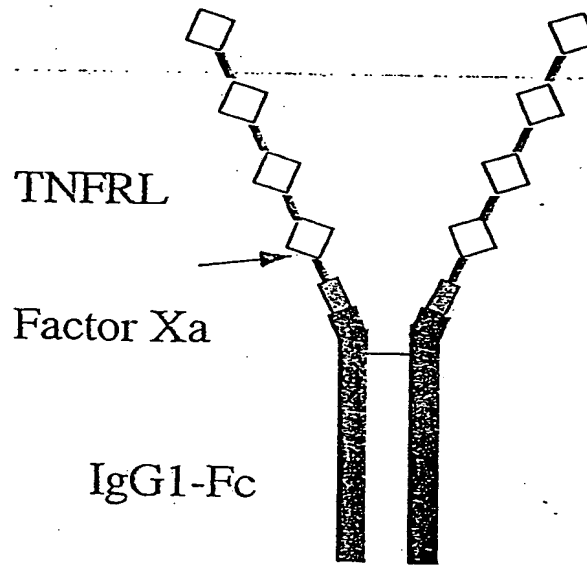


Figure 6. Schematic Representation of the sTNF-RSS Domain Fold (See also Figures 4 and 5.) The three disulfide bridges are represented by very thick lines and the structurally conserved parts by thick lines. (Here residues 55-60, 70-76, and 83-97 of domain 2 are taken as conserved; these superpose on domain 3 with an RMS error of 0.98 Å.) In the nonconserved regions, the domain 2 structure is shown as a medium line and the domain 3 structure as a thin line. The N- and C-terminal domains are marked N and C, respectively; the arrows are a reminder that the interdomain connections are not conserved. The second α -strand from loop 3 to the C-terminus carries the conserved Asp/Asn-Thr-Val motif, indicated by D, T, and V, and runs behind the down-strand. The conserved Ser is marked S, and the three strand-bridging hydrogen bonds it makes to D and T are shown as dotted lines. A β meander lies in front of the V and the third disulfide bridge. The conserved Tyr/Phe is indicated by F. It reaches from loop 1 behind the second disulfide bridge and packs against the conserved Asp/Asn to Ser bridge (D-S). The β turn positions are indicated by β .

Receptor-IgFc fusions



COOMASSIE STAINED SDS-PAGE GEL ANALYSIS OF 8UG TNFRL2Fc UNDER REDUCING AND NON-REDUCING CONDITIONS

<u>Non</u> <u>Reduced</u>	<u>Reduced</u>
200kD -	200kD -
93kD -	93kD -
69kD -	69kD -
46kD -	46kD -
30kD -	30kD -
22kD -	22kD -

BESTFIT of: Stnfr11.Seq check: 8473 from: 1 to: 1173

REFORMAT of: Stnfr11.Seq check: -1 from: 1 to: 1173 May 9, 1995 10:28
Coding region of soluble TNFR pulled from ATG full length report
80245.000, project ID: HSABH13 (full length clone = 195197)

to: 195197contigedtext.Seq check: 4750 from: 1 to: 1850

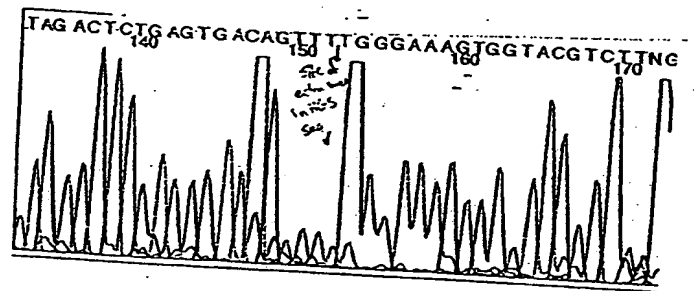
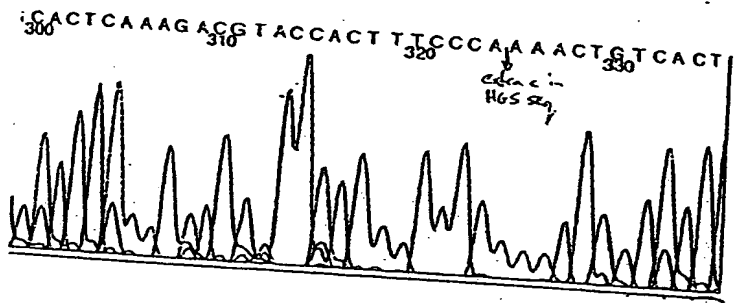
Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Swgapdna.Cmp
CompCheck: 5234

Gap Weight: 5.000 Average Match: -1.000
Length Weight: 0.300 Average Mismatch: -0.900
Quality: 1166.7 Length: 1173
Ratio: 0.995 Gaps: 1
Percent Similarity: 100.000 Percent Identity: 100.000

Stnfr11.Seq x 195197contigedtext.Seq October 17, 1995 16:21 ..

```

*ATENT 951 GGCATGCAAACCCAGTGACCAGATCCTGAAGCTGCTCAGTTGTGGCGAA 1000
          |||||||
S 1270 GGCATGCAAACCCAGTGACCAGATCCTGAAGCTGCTCAGTTGTGGCGAA 1319
          |||||||
1001 TAAAAATGGGACCAAGACACCTTGAAGGGCTAATGCACGCACTAAAG 1050
          |||||||
1320 TAAAAATGGGACCAAGACACCTTGAAGGGCTAATGCACGCACTAAAG 1369
          |||||||
1051 CACTCAAGACGTACCACTTTCCCACTGCTCACTCAGAGTCTAAAGA 1100
          |||||||
1370 CACTCAAGACGTACCACTTTCCCACTGCTCACTCAGAGTCTAAAGA 1418
          |||||||
1101 AGACCATCAGGTTCTTTCACAGCTTCAATGTACAAATTGTATCAGAAG 1150
          |||||||
1419 AGACCATCAGGTTCTTTCACAGCTTCAATGTACAAATTGTATCAGAAG 1468
          |||||||
1151 TTATTTTGTAGAAATGATAGGTA 1173
          |||||||
1469 TTATTTTGTAGAAATGATAGGTA 1491
          |||||||
```



TRANSLATE of: stnfrlikel.seq .eck: 8473 from: 1 to: 1173
generated symbols 1 to: 391.

REFORMAT of: Stnfrlikel.Seq check: -1 from: 1 to: 1173 May 9, 1995 10:28
(No documentation)

Stnfrlikel.pep Length: 391 May 9, 1995 16:13 Type: P Check: 8773 ..

RIENT 1 MALLCCALV FLDISIKWTT QETFPKYLH YDEETSHQLL CDKCPGTYL
51 KQCTAKWKT VCAPCPDHYI TDSWHTSDEC LYCSPVCKEL QYVQECNRT
101 HNRVCECKEG RYLEIEFCLK HRSCPPGFGV VQAGTPERNT VCKRCPDGFF
151 SNETSSKAPC RKHTNCSVFG LLLTQKGNAT HDNICSNGSE STQKCGIDVT
201 LCEEAFFRFA VPTKFTPNWL SVLVDNLPQT KVNRESVERI KRQHSSQEQT
251 FQLLKLWKHQ NKDQDIVKKI IQDIDLCENS VQRHIGHANL TFEQLRSLME
301 SLPGKKVGAE DIETIKACK PSDQILKLLS LWRIKNGDQD TLKGLMHALK
351 HSKTYHFPIN CHSESKEDHQ VPSQLHNQVI VSEVIFRNDR *

TRANSLATE of: 195197contigedtext.seq check: 4750 from: 320 to: 1525
generated symbols 1 to: 402.

195197contigedtext.Pep Length: 402 October 17, 1995 17:01 Type: P Check: 5187 ..

3CLONE 1 MALLCCALV FLDISIKWTT QETFPKYLH YDEETSHQLL CDKCPGTYL
51 KQCTAKWKT VCAPCPDHYI TDSWHTSDEC LYCSPVCKEL QYVQECNRT
101 HNRVCECKEG RYLEIEFCLK HRSCPPGFGV VQAGTPERNT VCKRCPDGFF
151 SNETSSKAPC RKHTNCSVFG LLLTQKGNAT HDNICSNGSE STQKCGIDVT
201 LCEEAFFRFA VPTKFTPNWL SVLVDNLPQT KVNRESVERI KRQHSSQEQT
251 FQLLKLWKHQ NKDQDIVKKI IQDIDLCENS VQRHIGHANL TFEQLRSLME
301 SLPGKKVGAE DIETIKACK PSDQILKLLS LWRIKNGDQD TLKGLMHALK
351 HSKTYHFPKT VTQSLKKTIR FLHSFTMYKL YQKLFLEHIG NQVQSVKISC
401 L*

PY10

```

PATENT 1 MNKGLLOCALVFLDISIKWTIQETFPFKLYLYDEETSHQLLCDCKCPGTYL 50
      |||
JB CLONE 1 MNKGLLOCALVFLDISIKWTIQETFPFKLYLYDEETSHQLLCDCKCPGTYL 50
      |||
51 KQHCTAKWKTVCA PCPDHYTDSWHTSDECLYCSFVCKELQYVQKQECNRT 100
51 KQHCTAKWKTVCA PCPDHYTDSWHTSDECLYCSFVCKELQYVQKQECNRT 100
      |||
101 HNRVCECKEGRYLEIEFCLKHRS CPPGFGVVQAGTPERNTVCKRC PDGFF 150
101 HNRVCECKEGRYLEIEFCLKHRS CPPGFGVVQAGTPERNTVCKRC PDGFF 150
      |||
151 SNETSSKAPCRKHTNCSVFGLLLTQKGNATHDNICSGNSESTQKCGIDVT 200
151 SNETSSKAPCRKHTNCSVFGLLLTQKGNATHDNICSGNSESTQKCGIDVT 200
      |||
201 LCEEAFFRFVPTKFTPNWLSVLVDNLPGTKVNAESVERIKRQHSSQBOT 250
201 LCEEAFFRFVPTKFTPNWLSVLVDNLPGTKVNAESVERIKRQHSSQBOT 250
      |||
251 FQLLKLWKHQNKDQDIVKIIQIDIDL CENS VQRHIGHANLTFEQLRSLME 300
251 FQLLKLWKHQNKDQDIVKIIQIDIDL CENS VQRHIGHANLTFEQLRSLME 300
      |||
301 SLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIRKNGDQDTLKG LMHALK 350
301 SLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIRKNGDQDTLKG LMHALK 350
      |||
351 HSKTYHFPTRCHSESKED.....HQVP SOLHNVOIVS 382
      |||
351 HSKTYHFPKTVTQSLKKTIRFLHSFTMYKLYQKLFLENTGNQVQSVKSK 400
      |||

```

Ligands

1. Fas ligand-like

HTPAN08

I, Protein was purified from E. coli. expression system.

II, Polyclonal antibody was made.

III, Expression and purification of protein from baculovirus is in progress.

VI, Expression in COS and CHO cells is in progress.

V, Functional study is in progress.

Ligands

2. TNF-like (TNF gamma)

HUVEO91

I, Two new constructs with different N-terminal sequences (no tag) were made, and expressed in E. coli. Protein was purified.

II, Functional Study is in progress.

III, Polyclonal and monoclonal antibodies are not available at present.

VI, Expression in COS and CHO cells is in progress.

V, Expression level of protein in baculovirus is low.

TNF Ligand, Receptor and Receptor Associated Factor Superfamily

I, Overview

II, Class I TNF Ligand, Receptor and Receptor Associated Factor Superfamily Members in HGS Database

III, Novel TNF Ligand, Receptor and Receptor Associated Factor Superfamily Members in HGS Database

<u>Ligands</u>	<u>Receptors</u>	<u>Receptor Associated Factors</u>
1, TNF α	TNFR1	FADD (MORT1), TRADD, TRAP-1, TRAP-2, TRAK, RIP,
2, LT α	TNFR2	EB16 (TRAF1), TRAF2 (TRAP-3), TRAF3 (CD40bp, LAP-1, CRAF-1, CAP-1)
3, LT β	TNFR-RP (LT β R)	EB16 (TRAF1)
4, FasL	Fas (APO-1)	FADD (MORT1), TRADD, RIP, FAP-1, FAST
5, CD40L	CD40	TRAF3 (CD40bp, LAP-1, CRAF-1, CAP-1) TRAF2 (TRAP-3), EB16 (TRAF1)
6, CD30L	CD30	??
7, CD27L	CD27	??
8, 4-1BBL	4-1BB	??
9, OX40L	OX40	??
10,	NGFR	??
11,	PV-T2 (MyxomaT2)	??
12,	PV-A53R	??
13,	ECPI	??
14,	Cowpox CrmB	??
15,	VVsaIF19R	??
16,	SFVT2	??
17,	G4R	??
18,	C22/B28R	??
19,	G2R	??

TNF Signal transducers

I, Receptor Associated Factors:

- 1, EB16 (TRAF1),
- 2, TRAP-3 (TRAF2),
- 3, TRAF3 (CD40bp, LAP-1, CRAF-1, CAP-1, TRAF2)
- 4, FADD (MORT1),
- 5, TRADD,
- 6, TRAP-1,
- 7, TRAP-2,
- 8, RIP

II, Protein Tyrosine Phosphatases :

FAP-1

III, Protein Tyrosine Kinases:

- 1, TRAK
- 2, FAST

**Class I TNF Ligand, Receptor and
Receptor Associated Factor Superfamily
Members in HGS Database**

Ligands

1, TNF α	Yes, Full length,	HMPAD01
2, LT α	Yes, Full length,	HTAAU17
3, LT β	Yes, Full length,	HNHCT93
4, FasL	Yes, Full length,	HNFCO16
5, CD40L	Yes, -320bp,	HLQAV55R
6, CD30L	Yes, -1000bp,	HSAVS23
7, CD27L	Yes, Full length,	HRGSD45
8, 4-1BBL	??,	??
9, OX40L	Yes, Full length,	HEMDB62

Receptor Associated Factors

1, EB16 (TRAF1)

TRAF1(mouse)	Cell Aug. 26 1994
EB16 (human)	Nov. 28, 1994 (Submitted), CellFeb. 10 1995
<u>HNFD065 (HEMCL41)</u>	<u>Identified during Sept. 1994</u>

2, TRAP-3 (TRAF2)

TRAF2 (mouse)	Cell Aug. 26 1994
TRAP3 (human)	Mar 2 1995, deposited in GenBank, unpublished
<u>HMWCE69</u>	<u>Identified during Sept. 1994</u>

3, TRAF3 (CD40bp, LAP1, CRAF-1, CAP-1)

TRAF2 (mouse)	Cell Aug. 26 1994
CD40bp (human)	Sept. 22 1994 (submitted) JBC Dec. 2 1994
LAP1 (human)	Nov. 28,1994 (Submitted), Cell Feb. 10 1995
CRAF-1 (human)	Science 10 1995
CAP-1 (human)	FEBS-Lett Jan 23 1995
<u>HTXFY28</u>	<u>Identified during Sept. 1994</u>

4, TRAP-1	Yes, Partial,	HMEGG76
-----------	---------------	---------

5, TRAP-2	Yes, Full length,	HSLCL12
-----------	-------------------	---------

Receptors

1, TNFR1	Yes, Full length,	HSLAB61
2, TNFR2	Yes, Full length,	HMEAE09
3, TNFR-RP (LT β R)	Yes, Full length,	HOUEL78
4, Fas (APO)	Yes, Full length,	HSBAW14
5, CD40	Yes, Full length,	HCABR73
6, CD30	Yes, ,-1280bp	HSEBA84
7, CD27	Yes, Full length,	HTTCB46
8, 4-1BB	Yes, Full length,	HTXER26
9, OX40	Yes, Full length,	HT5AI79
10, NGFR	Yes, -900bp	HCE1D55

Receptor Associated Factors

6, FADD (MORT1) Yes, Full length, HMEFX49, HUVCS86

FADD Feb. 23 1995 (Submitted) Cell May 19, 1995

MORT1 Jan 30 1995 (Submitted) JBC April 7, 1995

HUVCS86 Identified by death domain Jan 10 1995

7, RIP ??

8, TRADD Yes, Full, Splicing HT5AJ62, HT5AD11

9, FAP-1, Yes, Partial, HTEKR54

10, TRAK ??

11, FAST Yes, Full length, HODDG78

12, MLN62 Aug. 22 1995 (Deposited in GenBank, unpublished)

HTXAF08 (HRGB064) Identified on Nov. 1994

Novel TNF Ligand, Receptor and
Receptor Associated Factor Superfamily
Members in HGS Database

1

— 116

Ligands

I. Full length genes:

1, Fas ligand-like	HTPAN08
2, TNF-like (TNF gamma)	HUVE091
3, TNF-like (TNF delta)	HLTBT71
4, TNF-like (TNF epsilon)	HPDD012

II. Genes are not full length

1, Lymphotoxin-beta-like	HTGAK40
2, Fas ligand-like	HYBAG72
3, Fas ligand-like	HEMBK77
4, Fas ligand-like	HBNAZ12
5, Lymphotoxin-like	HATAF70
6, Lymphotoxin-like	HE8CL19
7, CD30L-like	HKPAB54
8, CD30L-like	HHPFK35

Receptors

I. Full length genes

1, TNFR p75-like	HHFFT79
2, TNFR p75-like	HMSBZ59
3, TNFR p75-like	HT1SB52
4, TNFR p75-like	HSABH13

II. Genes are not full length

1, TNFR p55-like	HTTBN61
2, TNFR p75-like	HHFUB83
3, TNFR p55-like	HFVGO43
4, OX40-like	HRDBI28
5, OX40-like	HTXBO18
6, Fas-like	HCAAA38
7, CD40-like	HSIED66
8, CD40-like	HTXBS40
9, CD40-like	HELAO61
10, CD40-like	HETDB40
11, CD27-like	HCEFL28
12, CrmB-like	HDSAP50
13, 4-1BB-like	HOFMB09

Receptor Associated Factors

I. Full length genes:

1, TRAF2 (TRAP-3)	HMWCE69
2, TRAF2-like (MLN62)	HTXAF08X
3, TRADD splicing variant	HT5AJ62RA
4, EB16 (TRAF1)	HNFD065
5, TRAF3	HTXFY28
6, FADD	HUVCS86

II. Genes are not full length:

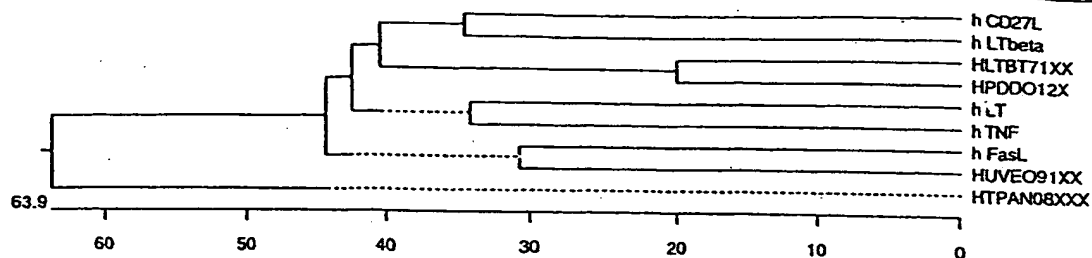
1, FADD-like	HHFHY89
2, TRAF-like	HE2PH69
3, TRAF-like	HETAP39
4, TRAF-like	HLMCT93
5, TRAF-like	HL2AH30
6, CD40bp-like	HUVCY67
7, TRAF-like	HLHDL86
8, TRAF-like	HLHCM30
9, TRAF-like	HJTAC77

- V A H - V I - - P - - Q - - - L L - - - - - W E - - - L R R G R A L L A S G V										Majority
170 180 190 200										
69	-	-	-	-	-	-	-	-	-	h CD27L
145	K	V	A	H	-	L	T	G	S	h FasL
63	P	A	A	H	-	L	I	G	D	h LT
58	P	A	A	H	-	L	I	G	A	h LTbeta
66	P	V	S	H	-	D	V	A	N	h TNF
34	-	-	-	-	-	-	-	-	-	HUVE091XX
133	-	V	T	E	-	-	-	-	-	HLTST71XX
52	-	V	T	E	-	-	-	-	-	HPDD012X
121	G	R	S	N	-	T	L	S	S	HTPAN08XXZ
E L S N C E - L V I P E A C L Y L V Y S Q V L F R G Q - - - C - - - - S - -										Majority
210 220 230 240										
90	E	L	D	K	G	Q	-	-	-	h CD27L
175	K	Y	K	K	G	G	-	-	-	h FasL
93	S	L	S	N	S	-	-	-	-	h LT
118	Q	F	S	D	A	E	G	A	L	h LTbeta
118	E	L	D	K	G	Q	-	-	-	h TNF
56	N	Y	T	H	K	E	-	-	-	HUVE091XX
154	-	-	-	-	-	-	-	-	-	HLTST71XX
73	-	-	-	-	-	-	-	-	-	HPDD012X
158	H	L	R	E	G	E	-	-	-	HTPAN08XXZ
- S R H V - L T H S H G Q V V S K - G - G Y P E T L L L L I S S K S S C P D R A										Majority
250 260 270 280										
120	A	S	R	H	-	-	-	-	-	h CD27L
207	L	S	H	K	O	Y	M	R	N	h FasL
125	T	S	S	P	L	Y	-	-	-	h LT
153	Q	G	R	S	Y	T	R	S	S	h LTbeta
147	-	-	-	-	-	-	-	-	-	h TNF
50	Q	A	G	R	P	N	K	P	D	HUVE091XX
174	-	-	-	-	-	-	-	-	-	HLTST71XX
93	-	-	-	-	-	-	-	-	-	HPDD012X
196	I	K	E	N	T	K	N	D	K	HTPAN08XXZ
- - - - - W Y N S I Y L G G V F Q L T Q G D R L S V N I S R A S L V D - -										Majority
290 300 310 320										
156	Q	R	-	-	-	-	-	-	-	h CD27L
237	-	-	-	-	-	-	-	-	-	h FasL
158	-	-	-	-	-	-	-	-	-	h LT
153	R	R	Q	Y	G	P	L	-	-	h LTbeta
182	P	E	G	A	E	A	K	P	-	h TNF
130	N	-	-	-	-	-	-	-	-	HUVE091XX
208	-	-	-	-	-	-	-	-	-	HLTST71XX
127	-	-	-	-	-	-	-	-	-	HPDD012X
235	A	E	Y	G	L	-	-	-	-	HTPAN08XXZ

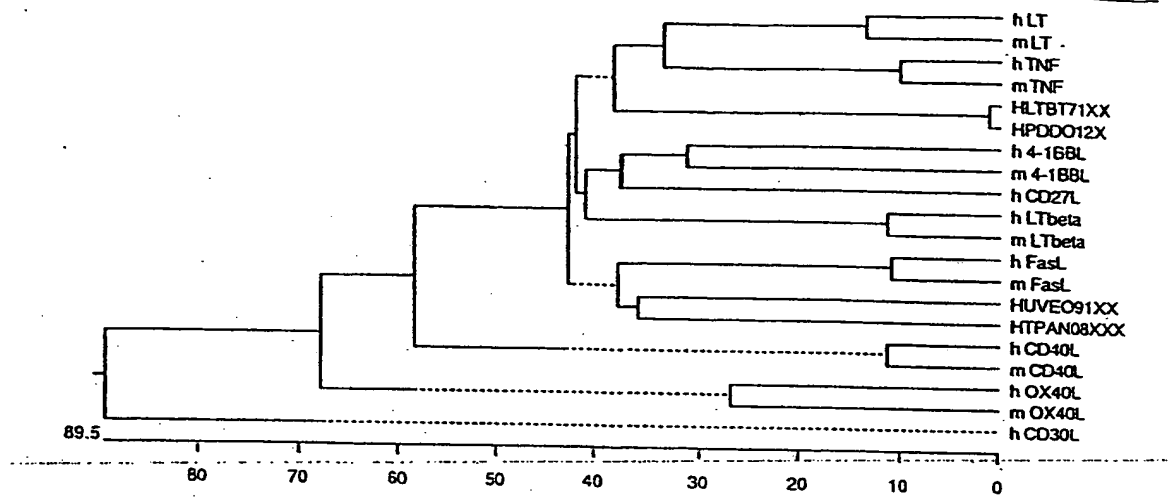
TAIL 4

- F A S S C G T F F G A V K - V - L															Majority		
330																	
178	-	S	R	N	T	D	E	F	F	G	V	Q	W	-	V R P		
269	-	F	E	E	S	-	Q	I	T	S	G	L	Y	R	- -	L	h CD27L
193	-	L	S	P	E	T	-	V	T	G	A	F	A	-	-	L	h FasL
231	-	F	A	R	-	S	K	F	T	F	G	A	V	M	-	V G	h LT
220	-	F	A	E	S	G	Q	V	T	F	G	I	A	-	-	L	h LTbeta
161	-	Y	T	K	E	D	K	F	T	F	G	A	V	L	-	L	h TNF
237	-	N	L	S	P	R	G	A	F	L	G	F	V	R	-	L	HUVZ091XX
156	-	N	L	S	P	R	G	A	F	L	G	F	V	R	-	L	ELTB711XX
268	-	M	D	H	E	A	S	-	F	F	G	A	V	L	-	V G	EPD0012X
																	HTPAN08XXX

Decoration 'Decoration #1': Shade (with solid bright yellow) residues that match the Consensus exactly.



tn16

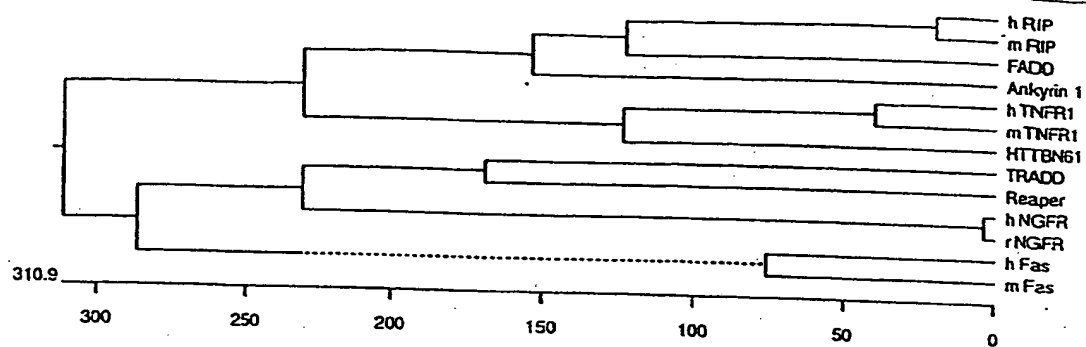


	V I D E										G - W K E F A R E L										G L S E S E I										Majority																	
	10					20					30					40																																
1	-	N	R	P	L	S	L	K	D	Q	O	T	F	A	R	S	V	G	L	K	M	R	K	V	G	F	S	L	Q	R	G	C	R	A	P	R	D	P	A	L	T	R	A	D	O			
1	L	C	A	A	F	N	V	I	C	D	-	-	-	-	N	V	G	L	K	M	R	R	R	L	P	R	Q	P	-	-	-	-	-	K	V	S	D	T	K	I	F	A	D	O				
1	H	A	V	A	F	Y	I	P	D	O	A	T	L	L	R	E	A	E	G	K	E	Q	O	I	L	E	-	-	-	-	-	-	-	I	R	E	S	Q	W	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	V	I	R	E	-	-	-	-	H	L	G	L	S	N	A	E	L	P	R	E	T	-	-	-	-	-	-	Q	F	S	V	E	D	-	-	-	-	-	-	-	-	
1	I	A	G	V	M	T	L	S	Q	V	-	-	-	-	H	L	G	L	S	-	K	G	F	V	F	K	N	-	-	-	-	-	G	V	N	S	A	K	H	-	-	-	-	-	-	-	-	
1	I	A	E	D	M	T	I	O	E	A	-	-	-	-	-	-	-	-	-	-	K	K	F	E	S	E	N	-	-	-	-	-	N	I	K	E	C	K	H	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	I	R	E	-	-	-	-	-	N	L	G	K	H	-	N	C	A	F	K	E	L	-	-	-	-	-	-	G	F	T	Q	S	Q	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	I	R	E	-	-	-	-	-	N	L	G	R	Q	-	N	C	A	F	K	E	L	-	-	-	-	-	-	G	F	T	Q	S	Q	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	V	V	E	N	-	-	-	-	-	-	-	-	-	-	V	P	L	R	-	-	-	-	-	-	-	-	-	G	F	T	Q	S	Q	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	V	V	D	G	-	-	-	-	-	-	-	-	-	-	V	P	P	A	R	-	-	-	-	-	-	-	-	G	F	T	Q	S	Q	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	R	H	L	A	G	E	H	-	-	-	-	-	G	F	T	Q	S	Q	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	R	H	L	A	G	E	H	-	-	-	-	-	G	F	T	Q	S	Q	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	V	M	D	A	-	-	-	-	-	-	-	-	-	-	V	P	A	R	R	-	-	-	-	-	-	-	-	G	F	T	Q	S	Q	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	F	T	Q	S	Q	-	-	-	-	-	-	-	-	-	

[illegible]

	L R K I Q R - A D L V N I L -		Majority
	90		
74	- - - - - - - - R L V		TRADD
69	L R S C M - N L - V A D L V		TRADD
61	- - - - - - - - R K P S Q		Reaper
63	L R N I D R - S E - I V T H M E		Ankyrin 1
63	L K E A N L C T L A - E K I Q T		b Fas
63	L K K A E - C R R T - D K F Q D		m Fas
64	L H Q C S R - I D - L L S S E		a RIP
64	L H Q C C R - I D - L L L H E I		m RIP
64	L R D M D L - L G C L E D H E E		b TNFR1
64	L S R M N L - A G C L E E I E		m TNFR1
47	L R R I O R - A D - L A		b NGFR
47	L R R I O R - A D - I U		x NGFR
60	W S A L		HTTENG1

Decoration 'Decoration #1': Shade (with solid bright yellow) residues that match the Consensus exactly.



MLC-----LLW-PL-LGL-L-LAAS-----PG-P-----Majority

10 20 30 40

1 MLCVLPGLVGLSATPA----- h CD27 Protein
1 MLCVLPGLVGLSATPA----- h LTbetaR Protein
1 MLCVLPGLVGLSATPA----- h NGFR (Ia) Protein
1 MLCVLPGLVGLSATPA----- h OK430 Protein
1 MLCVLPGLVGLSATPA----- h TNFR I Protein
1 MLCVLPGLVGLSATPA----- h TNFR II Protein
1 MLCVLPGLVGLSATPA----- h CD30 Protein
1 MLCVLPGLVGLSATPA----- h CD40 Protein
1 MLCVLPGLVGLSATPA----- h 4-1BB Protein
1 MLCVLPGLVGLSATPA----- h Fas protein
1 MLCVLPGLVGLSATPA----- h HT15B52XX
1 MLCVLPGLVGLSATPA----- h HXFF779XX
1 MLCVLPGLVGLSATPA----- h HX5Z59XX
1 MLCVLPGLVGLSATPA----- h HSAH13XX

-----TVLTC-----YLTSGQ-CCSACPPGTFLVQ Majority

50 60 70 80

24 MLCVLPGLVGLSATPA----- h CD27 Protein
36 MLCVLPGLVGLSATPA----- h LTbetaR Protein
29 MLCVLPGLVGLSATPA----- h NGFR (Ia) Protein
27 MLCVLPGLVGLSATPA----- h OK430 Protein
36 MLCVLPGLVGLSATPA----- h TNFR I Protein
32 MLCVLPGLVGLSATPA----- h TNFR II Protein
26 MLCVLPGLVGLSATPA----- h CD30 Protein
24 MLCVLPGLVGLSATPA----- h CD40 Protein
17 MLCVLPGLVGLSATPA----- h 4-1BB Protein
32 MLCVLPGLVGLSATPA----- h Fas protein
15 MLCVLPGLVGLSATPA----- h HT15B52XX
15 MLCVLPGLVGLSATPA----- h HXFF779XX
27 MLCVLPGLVGLSATPA----- h HX5Z59XX
31 MLCVLPGLVGLSATPA----- h HSAH13XX

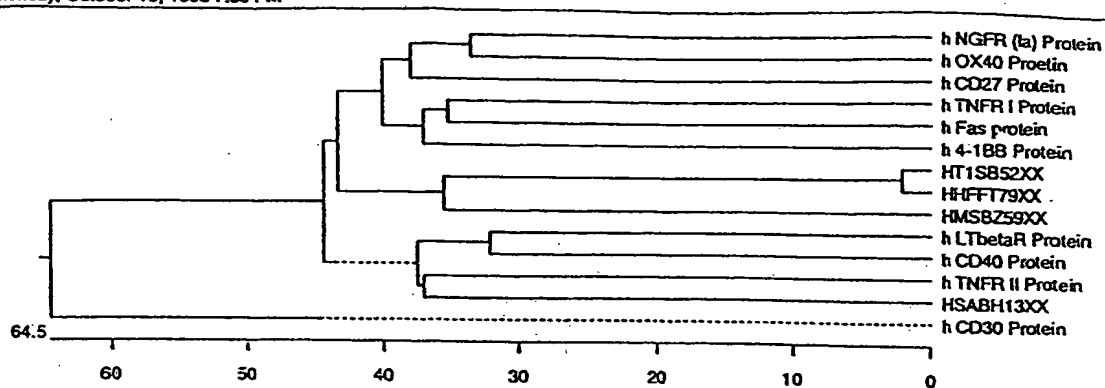
DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL Majority

90 100 110 120

52 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h CD27 Protein
71 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h LTbetaR Protein
56 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h NGFR (Ia) Protein
55 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h OK430 Protein
71 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h TNFR I Protein
66 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h TNFR II Protein
57 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h CD30 Protein
50 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h CD40 Protein
37 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h 4-1BB Protein
72 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h Fas protein
41 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h HT15B52XX
41 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h HXFF779XX
61 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h HX5Z59XX
53 DCTENEDTV-C-PCCGSSSYTD-WN-CTKCL-CS--CDGL h HSAH13XX

[illegible]

	210	220	230	240	Majority
105	-	-	-	-	-
179	-	-	-	-	h CD27 Protein
149	-	-	-	-	h LTbetaR Protein
128	-	-	-	-	h NGFR (Ia) Protein
167	TCHAGFFLREN	ECVSCSNCKKSLE	-	CTKLCIPQIE	h OK40 Protein
164	-	-	-	-	h TNFR I Protein
160	CASPENCK	EPSSGTIPQAKPTFVSPATSSASTMPVRC	-	-	h TNFR II Protein
146	-	-	-	-	h CD30 Protein
112	-	LTKKGCKDC	-	CFGTFN	h CD40 Protein
141	-	EHCDPCTKCEHGI	-	IKEC-TLTS	h 4-1BB Protein
192	-	-	-	-	h Fas protein
137	ESLSAEPVHAANGSVPLEPHARLSMASAPCGQAGLRRLRD	-	-	HTISRS2XX	HTISRS2XX
175	VTCLRPGLQGEACVPQITP	-	SHGPDKASSDLSFLEAQD	-	HMSFT79XX
145	-	-	-	-	HMSZ59XX
					MSAEH1XX



TNFR p55-like HTTBN61

Query= Manual - 0
(1111 letters)

gp|M63122|PATTNFR_1 tumor necrosis factor receptor ... +1 107 8.0e-20 5
gp|X59238|XMP53R_1 p55 tumor necrosis factor recep... +1 106 2.6e-17 4
>gp|M63122|PATTNFR_1 tumor necrosis factor receptor (Rattus norvegicus)
>pir|S|GQRTT1 tumor necrosis factor receptor type 1 precursor - rat
Length = 461

Score = 107 (49.2 bits), Expect = 8.0e-20, Sum P(5) = 8.0e-20
Identities = 21/36 (58%), Positives = 25/36 (72%), Frame = +1

Query: 913 LYDVMDAVPAREWKEFVRTXGLREAEIEAVEVEIGR 1020
LY V+D VP RWKEF+R GL E EIE +E++ GR
Sbjct: 355 LYAVVDGVPPTRWKEFMPILGLSEREIERLELQNGR 401

Score = 70 (32.2 bits), Expect = 8.0e-20, Sum P(5) = 8.0e-20
Identities = 13/32 (40%), Positives = 18/32 (55%), Frame = +1

Query: 52 RGTXLEKAPCTEPCGNSTCLVCPQDTFLAWENH 147
+GT L + C P - C VC + TP A +NH
Sbjct: 54 RGTYLVSDCPSPGQETVCEVCDXGTFASQNH 95

Score = 55 (25.7 bits), Expect = 2.0e-20, Sum P(5) = 2.0e-20
Identities = 11/32 (34%), Positives = 16/32 (50%), Frame = +1

Query: 157 AARPVNEQASQVALENCSAVRDTRCGCKXGWF 262
- - -- QV + C A DT CGCK F
Sbjct: 101 SCKTCRKMFPQVEISPCAKRQTVCGCKXGWF 132

Score = 45 (21.2 bits), Expect = 8.0e-20, Sum P(5) = 8.0e-20
Identities = 7/16 (43%), Positives = 10/16 (62%), Frame = +1

Query: 1024 RDOQVEMKRWPHKQP 1071
R- Y ML+ WR + P
Sbjct: 404 REAHVSMLEAWPRRT 419

Score = 39 (17.9 bits), Expect = 8.0e-20, Sum P(5) = 8.0e-20
Identities = 8/18 (44%), Positives = 10/18 (55%), Frame = +2

Query: 425 LPCPAPRAPWGAQSAV 478
+P PAP W V +A ?
Sbjct: 338 VPAPAPVRKWDVVAQ 355

Score = 39 (17.9 bits), Expect = 4.5e-17, Sum P(5) = 4.5e-17
Identities = 8/17 (35%), Positives = 9/17 (52%), Frame = +2

Query: 215 CSAVADTRCGCKXGWFV 265
C +T C C G+F+
Sbjct: 158 CKRQNTVCNCHAGFFL 174

Score = 38 (17.5 bits), Expect = 1.1e-05, Sum P(4) = 1.1e-05
Identities = 9/25 (36%), Positives = 10/25 (40%), Frame = +3

Query: 545 ANGHPEPTHTATAGLTSPWLLQMKL 620
AN P H AT + L M L
Sbjct: 413 ANRRRTPRHEATLDVVGAVLCNNL 437

Query= Manual - 0
(1111 letters)

Plus grand Espe:

```

Query:      254 GGCTGGTT 261
            |  ||
Subject:    463 AACCAATT 490

```

TNFR p55-like HFVGO43

>gp|M60468|MUSMTNFR1_1 murine tumor necrosis factor receptor 1 [Mus musculus]

Score = 60 (27.2 bits), Expect = 0.00066, Sum P(2) = 0.00066
Identities = 14/35 (40%), Positives = 19/35 (54%), Frame = +3

Query: 15 VTXPKTPTNHSPPSPASQTXGFSKPSFLLSVXSTEL 119
+T +P SP+S + T GFS P F V ST +

Sbjct: 264 LTPAPSPAFSPTSGFNPTLGFSTPGFSSPVSSTPI 298

Score = 37 (17.0 bits), Expect = 0.00066, Sum P(2) = 0.00066
Identities = 7/12 (58%), Positives = 9/12 (75%), Frame = +2

Query: 104 LLY*APCAVPXP 139

LLY + C+VP P

Sbjct: 325 LLYESLCSVPAP 336

>gp|K76656|USTNFR20S3_1 tumor necrosis factor receptor [Mus musculus]
Length = 454

Score = 60 (27.2 bits), Expect = 0.00066, Sum P(2) = 0.00066
Identities = 14/35 (40%), Positives = 19/35 (54%), Frame = +3

Query: 15 VTXPKTPTNHSPPSPASQTXGFSKPSFLLSVXSTEL 119
+T +P SP+S + T GFS P F V ST +

Sbjct: 264 LTPAPSPAFSPTSGFNPTLGFSTPGFSSPVSSTPI 298

Score = 37 (17.0 bits), Expect = 0.00066, Sum P(2) = 0.00066
Identities = 7/12 (58%), Positives = 9/12 (75%), Frame = +2

Query: 104 LLY*APCAVPXP 139

LLY + C+VP P

Sbjct: 325 LLYESLCSVPAP 336

HOFMB09

Plus Strand ESPs:

[illegible]

Score = 132 (35.5 bits), Expect = 1.5e-34, Sum P(2) = 1.5e-34
Identities = 30/35 (85%), Positives = 30/35 (85%), Strand = Plus / plus

```
>gc|U03397|HSU03397_1 4-168 [Homo sapiens]
      Length = 255
```

Plus Strand HSPs:

Score = 138 (63.2 bits), Expect = 5.0e-20, Sum P(2) = 5.0e-20
Identities = 25/36 (69%), Positives = 29/36 (80%), Frame = +1

Query: 160 MGSGYNNIVAALLVNFERTRSIQDSCSKCPVGDF 257
MG+ YNIVA +LLV+NFERTRS+QD CS CP G F
Sbjct: 1 MGNSCYNIVATLLLVNFERTRSLODPCSNCPAGTF 36

Score = 73 (33.4 bits), Expect = 5.0e-20, Sum P(2) = 5.0e-20
Identities = 11/23 (47%), Positives = 15/23 (65%), Frame = +3

Que-y: 300 PCPPNFSNTSGQNXVSYARRCE 368
PCPPN+FS+ GQ R+C+
Sbjct: 47 PCPPNSFSSAGGQRTCDICROCK 69

TABLE

CD40-like

HETDB40

>sp|X75962|HSEOX40MR_1 OX40 homologue {Homo sapiens}

Length = 277

Score = 52 (23.9 bits), Expect = 0.025, Sum P(3) = 0.026

Identities = 9/17 (52%), Positives = 9/17 (52%), Frame = +2

Query: 200 PYGSYNGSDCTRCAPG 250

P SY G DC CP G

Sbjct: 115 PLDSYKPGVDCAPCPG 131

Score = 52 (23.9 bits), Expect = 0.036, Sum P(3) = 0.035

Identities = 10/38 (26%), Positives = 16/38 (42%), Frame = +3

Query: 786 LEASDVGSSTSCPAGYYIDRDSGTCHSCTPTNTILKAH 899

L++ G C CP G++ D+ C T+ H

Sbjct: 116 LDSYKPGVDCAPCPGHHFSPGDNQACFPWTNCTLAGKH 153

Score = 51 (23.5 bits), Expect = 0.025, Sum P(3) = 0.026

Identities = 9/22 (40%), Positives = 11/22 (50%), Frame = +2

Query: 104 GAVKLLASGVKTHCPPCNPGFF 169

G.V + T C PC PGF+

Sbjct: 51 GAVSRCSRQNTVCRPCGPGFY 72

Score = 45 (20.7 bits), Expect = 0.22, Sum P(3) = 0.20

Identities = 7/14 (50%), Positives = 9/14 (64%), Frame = +2

Query: 188 CQPCPYGSYNGSD 229

C PCP G +S G +

Sbjct: 125 CAPCPPGHFSFGDN 138

Score = 44 (20.2 bits), Expect = 1.0, Sum P(3) = 0.64

Identities = 6/17 (35%), Positives = 12/17 (70%), Frame = +2

Query: 170 KTNKSTCQPCPYGSYSN 220

++ N+ C+PC G Y++

Sbjct: 58 RSQNTVCRPCGPGFYND 74

Score = 41 (18.9 bits), Expect = 0.026, Sum P(3) = 0.026

Identities = 9/22 (40%), Positives = 10/22 (45%), Frame = +3

Query: 615 PIPTSLRRTLPRASPGPSRGPL 680

PI PR S GPS P+

Sbjct: 186 PITVQPTAEPRTSQGPSTRPV 207

Score = 40 (18.4 bits), Expect = 0.036, Sum P(3) = 0.035

Identities = 7/30 (23%), Positives = 16/30 (53%), Frame = +2

Query: 176 NMSTCQPCPYGSYNGSDCTRCAPGTEPAV 265

++ C+PC ++ +GS+ + T+ V

Sbjct: 77 SSKPCKPCTWCNLRSGSERKQLCTATQDTV 106

Score = 36 (16.6 bits), Expect = 0.12, Sum P(3) = 0.12

Identities = 6/8 (75%), Positives = 7/8 (87%), Frame = +2

Query: 236 RCPAGTEP 259

RC AGT+P

Sbjct: 108 RCPAGTQP 115

Ligands

Cloning, Expression and Purification in Progress:

1, TNF-like (TNF delta) HLTBT71

Multiple tissue RNA blot analysis using HPDDO12 insert as probe showed that this gene is expressed in almost all the tissues tested. However, the expression in the heart was the highest followed by placenta and kidney.

2, TNF-like (TNF epsilon) HPDDO12

Receptors

I. HSABH13 TNFR p75-like

I, Protein was purified from baculovirus expression system.

II, Polyclonal antibody was made.

III, Functional study is in progress.

K.B. Tan
Alem Truneh
10.18.95

RNA Expression: TNF and TNFR Like Genes

TNF α	TNFI	TNFR1	TNFR2	TNFR3	OX40
413412	195197	103902	231556	117992	
HTPAN03	HSABH13X	HTXBS40	HTTBN61	HT4SD09	
ATG 343	ATG 348	ATG 363	ATG 338	ATG 342	
pancreatic	fetal lung	T cell	testis tumor	activated	
tumor	fibroblast line			T cells	
(class 1)	(class 3)	(class 2)	(class 2)	(class 5)	(class 1)

SUMMARY OF RNA EXPRESSION STUDIES

Gene	Myeloid cells	B cell	T cell	Osteo-sarcoma	Tissues	Primary cells	Multiple forms
TNF α	HL60	REH (pre B)			Heart	CD4 ⁺	
TNFI1 pancreatic tumor	KG1a		Jurkat		Heart Bone marrow	CD4 ⁺ CD19 ⁺	Yes
TNFI1 fetal lung fibroblast				MG63	Kidney		
TNFI2 T cell	KG1a		Jurkat		Lung, Thymus Spleen Bone marrow	CD4 ⁺ CD8 ⁺ CD19 ⁺	
TNFI3 testis tumor	KG1a				Thymus	CD4 ⁺	Yes
OX40 activated T cell	KG1a		Jurkat			CD4 ⁺	

DR3

RNA Expression: TNF and TNFR Like Genes

DR3

CELL LINES	TNF α (class 1)	TNFI1 413412 Pancreatic tumor (class 3)	TNFI1 195197 Lung fibroblast (class 2)	TNFI2 103902 T cell (class 2)	TNFI3 231556 Testis tumor (class 5)	OX40 117992 Activated T cells (class 1)
<i>Bone Marrow Stromal</i>						
TF274	-	\pm	+	-	-	-
<i>Osteosarcoma</i>						
MG63	-	-	++	-	-	-
HOS (TE85)		\pm	\pm	-	-	-
<i>Hematopoietic: Erythroid</i>						
K562			-			
<i>Hematopoietic: Myeloid</i>						
KG1a	-	++	-	+	++	+
KG1	-	++	-	\pm	+	\pm
PLB985			-		-	
HL60	++	\pm				
U937			-	\pm	-	
THP-1	\pm	\pm	\pm	+	-	-
<i>Hematopoietic: B-Lymphocyte</i>						
REH (Pre B)	+	-	-	\pm	-	-
BJAB (IgM)			-	-	-	
Raji (IgM)	\pm	-	-	\pm	-	-
IM-9 (IgG)			-	-	-	
<i>Hematopoietic: T-Lymphocyte</i>						
Sup-T1			-	-	-	
Jurkat	-	+	-	+	-	+
H9				+		
Molt-3	-	-	-	\pm	-	-
<i>Endometrial Carcinoma</i>						
RL95-2			-	-	-	
<i>Breast Cancer</i>						
MCF7	-	-	\pm	-	-	-
<i>Colon Cancer</i>						
BE			-	-	-	
HT29			-	-	-	
<i>Neuroblastoma</i>						
IMR32				-	-	

no entry = not tested, - = not detected, \pm to ++ = increasing amounts of RNA detected.

V02

RNA Expression: TNF and TNFR Like Genes DR3

TISSUES and CELLS	TNF α (class 1)	TNFI1 413412 HTPAN08 ATG 343 pancreatic tumor (class 3)	TNFR1 195197 HSABH13X ATG 348 fetal lung fibroblast line (class 2)	TNFR2 103902 HTXBS40 ATG 363 T cell (class 2)	TNFR3 231556 HTTBN61 ATG 338 testis tumor (class 5)	OX40 117992 HT4SD09 ATG 342 activated T cells (class 1)
HUMAN						
Brain	-	-	-	-	-	-
Heart	++	++	+	+	-	-
Lung	\pm	+	+	++	\pm	-
Thymus	-	-	-	++	++	-
Spleen	\pm	\pm	-	++	+	-
Liver	-	-	-	-	-	-
Kidney	-	+	++	+	-	-
Small Intestine	\pm	-	-	+	\pm	-
Prostate	-	\pm	-	+	-	-
Skeletal Muscle	\pm	-	-	-	-	-
Bone Marrow	\pm	++	-	++	\pm	-
PBL, CD19+	\pm	++	-	++	\pm	-
PBL, CD8+	\pm	+	-	++	-	-
PBL, CD4+ (activated)	+++	+++	\pm	++	++	++
RAT						
Brain	-	-	-	-	-	-
Heart	++	-	-	-	-	-
Lung	-	-	-	-	-	-
Thymus	-	-	-	-	-	-
Spleen	-	-	-	-	-	-
Kidney	+	-	-	-	-	-
Small Intestine	-	-	-	-	-	-

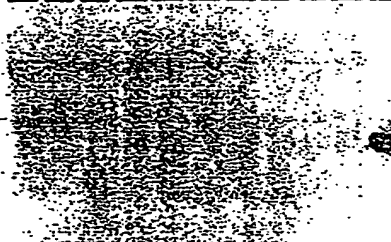
- = not detected, \pm to +++ = increasing amounts of RNA detected.

B H L T S L K I P M Turt



S. 363 403902 TNR L1

Brain Heart Lung Thymus Spleen Liver Kidney Intestine Prostate Muscle Turt



Tissue 1-13-85 C61 Amv66 0x40

B H L T S L K I P M Turt

285

185



S. 363 403902 TNR L2

Brain Heart Lung Thymus Spleen Liver Kidney Intestine Prostate Muscle Turt

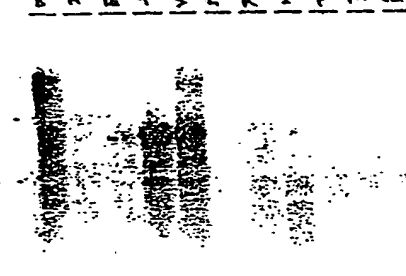


S. 363 403902 TNR L2

B H L T S L K I P M Turt

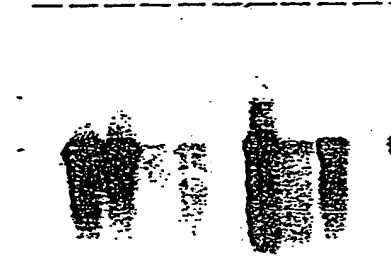
285

185

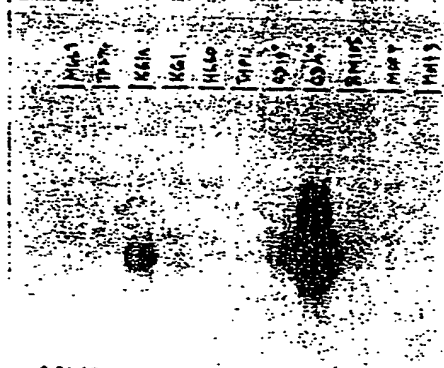
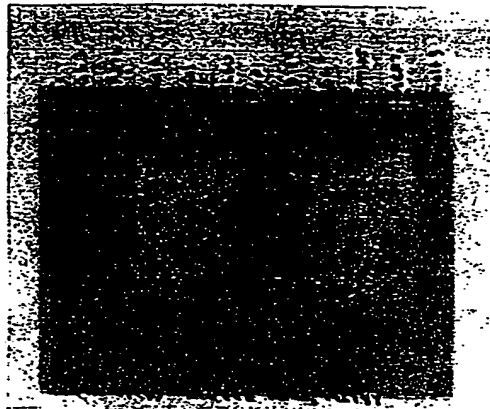


S. 338 231556 TNR L3

B H L T S L K I P M Turt

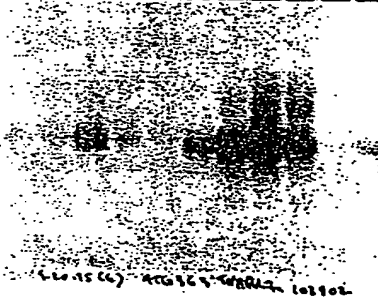


S. 343 413412 TNR L1



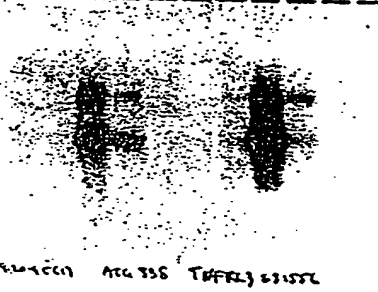
9-24-45 OK 40

M65
T234
K614
K61
H60
T234
C234
C234
B615
M65
M65



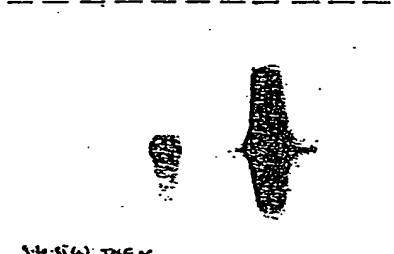
9-24-45 OK 40

M65
T234
K614
K61
H60
T234
C234
C234
B615
M65
M65



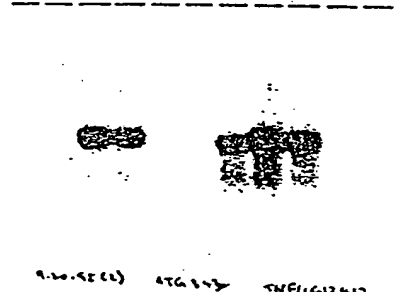
9-24-45 OK 40

M65
T234
K614
K61
H60
T234
C234
C234
B615
M65
M65



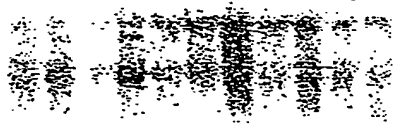
9-24-45 OK 40

M65
T234
K614
K61
H60
T234
C234
C234
B615
M65
M65



9-24-45 OK 40

RAT
Raji
Raji-Hu
REN
CD8
BM
Thymus
Spleen
Heart
Lung
Kidney
S. Intestine



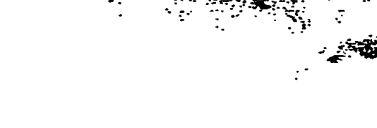
TNFRL3

RAT
Raji
Raji-Hu
REN
CD8
BM
Thymus
Spleen
Heart
Lung
Kidney
S. Intestine



TNFRL1

RAT
Raji
Raji-Hu
REN
CD8
BM
Thymus
Spleen
Heart
Lung
Kidney
S. Intestine



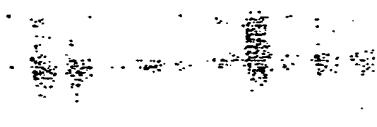
TNFRL2

RAT
Raji
Raji-Hu
REN
CD8
BM
Thymus
Spleen
Heart
Lung
Kidney
S. Intestine



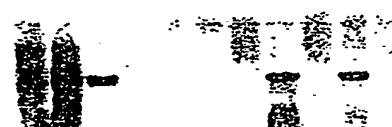
TNFRL3

RAT
Raji
Raji-Hu
REN
CD8
BM
Thymus
Spleen
Heart
Lung
Kidney
S. Intestine



OX40

RAT
Raji
Raji-Hu
REN
CD8
BM
Thymus
Spleen
Heart
Lung
Kidney
S. Intestine



TNFRL2

TNFRL2-Ig Binding to Cells

	Cell Line	Cell Lineage	Unstained	GAH-FITC Control	TNFRL2-Ig Binding
1)	THP1	Monocyte	2.2	5.2	51.0
	THP1 / LPS		2.3	5.4	53.1
2)	HL60	Pro-monocyte	2.0	5.2	138.3
	HL60 / PHA+PMA		2.4	10.6	171.8
3)	H9	T	2.1	6.3	14.3
	H9 / PHA+PMA		3.9	23.1	43.8
4)	Jurkat	T	2.1	3.6	19.4
	Jurkat / PHA+PMA		2.4	3.7	12.4
5)	KG1a	Myeloid Precursor	2.6	6.4	12.3
	KG1a / PHA+PMA		2.8	5.6	19.9

EXPRESSION OF TNF-LIKE PROTEIN

HGS clone 413412 = HTPAN08

Objectives:

- Express as fusion protein in *E. coli*--to be used for raising antibodies
- Express in soluble form in *E. coli* or other systems--to be used for receptor binding and activity assays

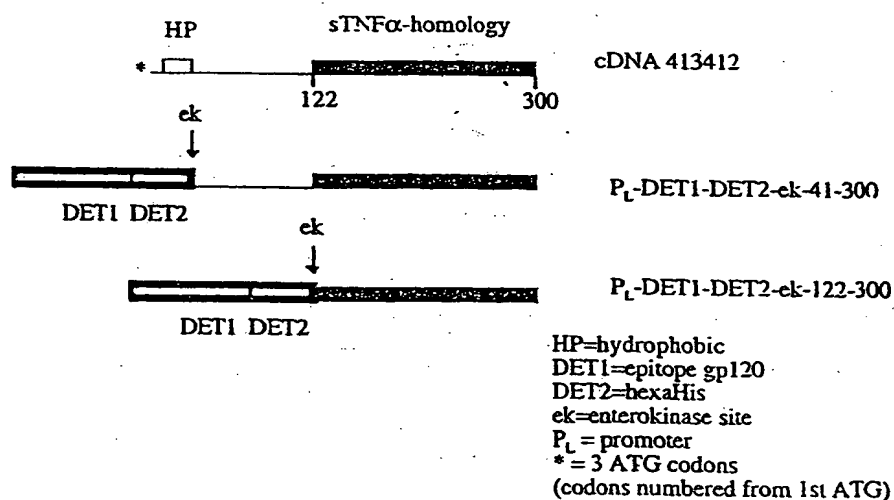
Accomplishments:

- Plasmid DNA from HGS strain (purified by K. B. Tan) was sequenced.

The DNA sequence in the open reading frame agreed with that reported by HGS.

There was a 1 bp difference upstream of the first of 3 closely spaced in-frame ATG codons, which makes it more likely that the first ATG is the translation initiator codon (TTCATGG in HGS sequence is ATCATGG in SB sequence; the latter is in good agreement with Kozak consensus upstream sequence)

- Two fusion constructs were made for *E. coli* expression; both expressed well as insoluble proteins; both have the potential for release of untagged protein following enterokinase digestion. Protein purification/rabbit injection is planned.



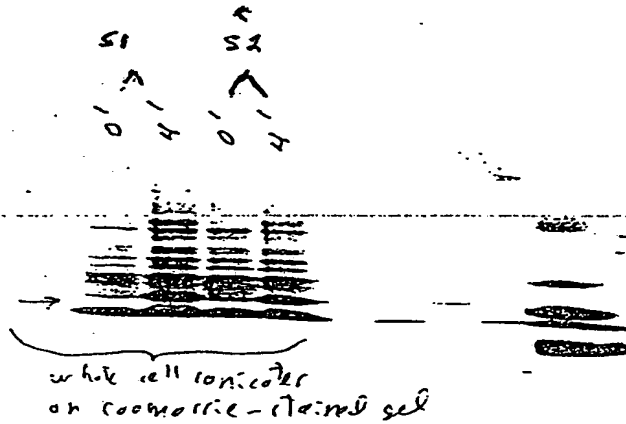
FBI

001

Expression of clone 413412 fusions in E. coli:

STRATAGENE EAGLEEVE II 10/16/95 14:59:23

IMAGE SIZE (640 x 480 x 81).
REAL-TIME ACQUIRE
IMAGE CREATED ON MON OCT 16 14:59:21 1995.

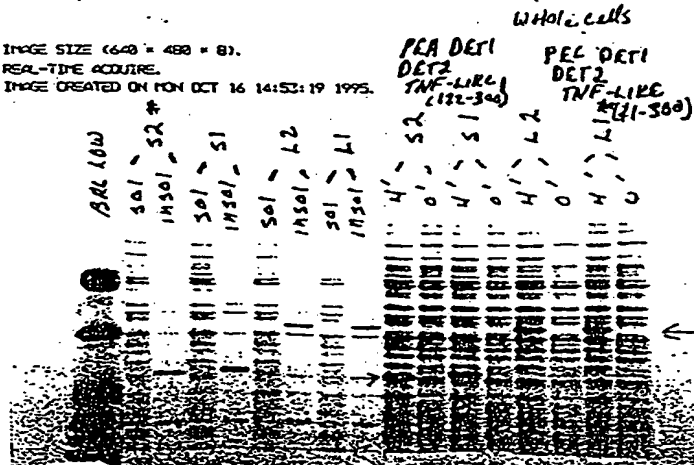


soluble and insoluble
fractions of cell lysates
on Coomassie stained gel

whole cell sonicates
on Coomassie-stained SDS-PAGE

STRATAGENE EAGLEEVE II 10/16/95 14:53:22

IMAGE SIZE (640 x 480 x 81).
REAL-TIME ACQUIRE
IMAGE CREATED ON MON OCT 16 14:53:19 1995.



FASTA of HSA VO45 vs Death Domain Proteins

The best scores are:

	init1	initn	opt
Sw:Tnr1_Human P19438 homo sapiens (human). tumor necrosi...	111	153	207
Sw:Tnr1_Mouse P25118 mus musculus (mouse). tumor necrosi...	113	144	208
Sw:Ank1_Human P16157 homo sapiens (human). ankyrin r (an...	47	47	55
Com\$94:[Hurlemr.Sequences.Pep]Rip.Pep mouse RIP	36	36	116
Sw:Fasa_Human P25445 homo sapiens (human). fasl receptor...	29	29	88
Sw:Ngfr_Human P08138 homo sapiens (human). low-affinity ...	28	28	37
Sw:Ngfr_Rat P07174 rattus norvegicus (rat). low-affinity...	28	28	37
Sw:Ngfr_Chick P18519 gallus gallus (chicken). low-affini...	28	28	37
Com\$94:[Hurlemr.Sequences.Pep]Fadd.Pep FADD/MORT1	27	27	29
Sw:Ankc_Human Q01485 homo sapiens (human). ankyrin, brai...	26	26	51
Com\$94:[Hurlemr.Sequences.Pep]Tradd.Pep TRADD	24	24	69
Sw:Fasa_Mouse P25446 mus musculus (mouse). fasl receptor...	22	22	33

	PR1	R1	R2	I1	R3	I3	R4	R5	I2	R6	I4	R7	PR7	
IHLHAB49	1	X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHTOAU65	3	X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHT1SB52	3	?	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHT0BH42	3	?	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHLHTJ50	1	X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHLMCG62		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHTOHT08		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHTXBS40		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHTXFP69		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHTXE024		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHLMAC26		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHMQCG18		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHEAAQ14		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHCUBW34		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHBNAQ20		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHOABR18		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHHEET79	1	X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHBNAQ51		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHT4SI80		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHMSBZ59		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHFNB43		X	X	X	X	X	X	X	X	X	X	X	X	X (full length)
IHROAG75	2	X	X	X	X	X	X	X	X	X	X	X	X	X (full length)

X (discontinuous ESTs)

X (discontinuous ESTs)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.